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Risk of Head and Neck Squamous Cell Cancer and Death in Transplanted and Untransplanted Patients with Fanconi Anemia

Short title: Risks in Transplanted and Untransplanted Patients with Fanconi Anemia

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Abstract

Hematopoietic stem cell transplant (SCT) is currently the only therapy that can restore normal hematopoiesis in patients with Fanconi Anemia (FA). FA patients have a high baseline risk of squamous cell cancers of the head, neck, and esophagus (SCC), and SCT conditioning may increase SCC incidence. We evaluated the risks of SCC and death in 145 untransplanted FA patients in the North American Survey (NAS) cohort, and 117 transplanted FA patients in the Hôpital Saint Louis (SLH) cohort. The age-specific hazard of SCC was 4.4-fold higher in transplanted versus untransplanted FA patients (P = 0.003), and SCC occurred at significantly younger ages in the former (respective medians: 18 and 33 years, P = 0.004). Survival after SCC was similarly poor in both cohorts (P = 0.135, median = 13 months). The hazard of SCC increased at a greater than linear rate, to 4.4%/y by age 40 in NAS and 4.7%/y by 10 years after transplant in SLH. In SLH, the hazard of non-SCC death was biphasic, declining significantly (P = 0.004) from 7.1%/month during the first six months after transplant to 0.13%/month (1.6%/y) after the first year. Acute and chronic graft versus host diseases were significant SCC risk factors. Adverse event rates in these cohorts provide historical control rates to assess emerging therapies for FA.

Key Words: Fanconi anemia, cancer, stem cell transplant, risk assessment

INTRODUCTION

Fanconi Anemia (FA) is an autosomal recessive genomic instability syndrome [1] associated with congenital abnormalities, progressive pancytopenia, and a predisposition to cancer [2]. Acute myeloid leukemia (AML) is the most frequent cancer of FA [3], but a number of specific solid tumors occur at remarkably high rates in FA patients who survive to adulthood, notably squamous cell cancers of the head, neck, and esophagus (SCC), and vulvar and cervical cancer in women [4,5].

Hematopoietic stem cell transplant (SCT) is currently the only therapy that can restore normal hematopoiesis in FA. It has been difficult to optimize transplant protocols for FA patients. Standard regimens are too toxic for FA patients [6,7], but the graft will fail if the conditioning is too mild. Effective regimens have been developed [8]. However, in non-Fanconi patient populations, conditioning increases the incidence of certain tumors [9-14], especially SCC, that occur at high baseline rates in FA. The occurrence of these cancers in Fanconi patients following SCT has been an ongoing concern [15-20].

Two methodological issues affect risk assessment in transplanted FA. First, SCT protocols for FA patients are institution-specific, and when protocols are enhanced over time, a number of modifications may be made at once. However, secondary solid tumors, and some transplant-related deaths, occur years after transplant. Therefore, the ultimate assessment of any new protocol must compare adverse event rates that manifest over time to corresponding rates in the past, i.e. using historical controls. Second, because the baseline risks of certain cancers are elevated in FA, the logical comparison group for transplanted FA patients is untransplanted FA patients. To our knowledge, this comparison has not previously been made.

In this study, we evaluate the risk of SCC and death in transplanted and untransplanted patients with FA. The transplant cohort consists of patients from Hôpital Saint Louis in France (SLH), one of the largest series of FA patients transplanted by a single team at a single institution [15,19,20]. The untransplanted cohort consists of patients in the North American Survey (NAS) [4], a retrospective natural history cohort with validated cancer diagnoses. Since transplant protocols for FA are being modified continuously, the adverse event rates in the SLH series may serve as historical control rates, and the patterns of risk in transplanted versus untransplanted patients may shed light on the etiology of tumors in FA.

MATERIALS AND METHODS

The Hôpital Saint Louis Transplant Cohort (SLH)

We studied 117 consecutive FA patients transplanted at the Hôpital Saint Louis in Paris, France (SLH) from November 1976 through October 2002 and followed through 15 February 2003. One half of the patients were transplanted during 1988 through 1997, and one quarter during 1998 through 2002. A single institutional team led by one of the authors (E.G.) transplanted all patients in the series. All treatment protocols were reviewed and approved by appropriate local institutional review boards in operation at the time of each transplant. Details of the protocols have been reported elsewhere [15,19,20].

This analysis investigates SCC and death rates in the entire cohort, incorporating recent follow-up. Treatment modalities have been refined over the study period. Most patients, (113 of 117, 97 percent), received cyclophosphamide 20 mg/kg, and most

patients (99 of 117, 85 percent) received irradiation. Seventy-three patients received total lymphoid irradiation at 5 Gy, and 26 patients received total body irradiation at 2 to 6 Gy. A number of other agents were used in the conditioning regimens of some patients, including anti-thymocyte globulin in 41 patients (35 percent, from 1979 to the present), busulphan in 10 patients (9 percent, beginning in 1994), and fludarabine in 13 patients (11 percent, beginning in 2000). CD34+ donor stem cell selection to effect T-cell depletion was performed in 18 transplants (15 percent, beginning in 1996 and ending in 1999). Prophylaxis for graft versus host disease included: cyclosporin A (CSA) alone (71 patients, 61 percent), CSA + monoclonal antibody (3 patients), steroids (3 patients), CSA + steroids (22 patients, 19 percent), methotrexate (5 patients), CSA + methotrexate (3 patients), CSA + anti-thymocyte globulin + steroids (10 patients, 9 percent).

Host factors, including the date of onset of acute or chronic graft versus host disease, and donor factors, including HLA match and donor sex, were extracted from the medical records. In this analysis, the 56 “matched” patients received stem cells from an HLA identical sibling. The 61 “unmatched” patients received stem cells from alternative donors, i.e. other relatives (9 patients) or unrelated donors (52 patients). An active effort was made to contact surviving patients near the end of the reporting period. Some patients could not be contacted, including a number of patients who had left France. A total of 15 patients (13 percent) were lost to follow-up prior to January 2000. Fifteen patients (13 percent) received a second transplant due to primary graft failure. We included follow-up beyond any second transplant in our analysis.

The North American Survey Natural History Cohort (NAS)

The North American Survey (NAS) is a retrospective natural history cohort of 145 persons with FA from the United States and Canada [4]. Cohort members belonged to the United States or Canadian FA family support groups and had proven FA by chromosome breakage analysis. The study database includes follow-up through October 2000. NAS subjects were followed for bone marrow failure leading to bone marrow transplant (BMT), AML, solid tumors, and death. Cancer diagnoses were confirmed using medical records, pathology reports, or death certificates. Fifty of the 145 NAS subjects were transplanted; 3 had prior AML and 2 had prior non-SCC solid tumors. In the remaining 45, the indications for BMT were aplastic anemia in 37 subjects in myelodysplastic syndrome in 8.

Statistical Methods

The most common solid tumor type in both cohorts was SCC; other tumors occurred too infrequently for meaningful comparative analysis. In both cohorts, survival after SCC (post-SCC Survival) could be evaluated. We compared the demographics of SLH and NAS using Fisher's exact test (proportions) or the Wilcoxon rank sum test (continuous variables). In each cohort, we estimated the effect of developing an SCC on overall survival using the Cox proportional hazards model [21], with the occurrence of SCC treated as a time-dependent covariate. We contrasted post-SCC Survival in SLH versus NAS using the Cox proportional hazards model, and estimated median post-SCC Survival using the actuarial (Kaplan-Meier) approach [22].

Competing Risks Analysis

Patients in each cohort were at risk of competing adverse events. In SLH, we analyzed development of SCC and non-SCC Death as competing risks; the time scale was years since transplant. In NAS, we analyzed development of SCC, non-SCC Death, and BMT as competing risks; the time scale was age in years. For comparability with SLH, the competing endpoints in this analysis of NAS differ from our previous report [4]. Here, we count BMTs in NAS that occurred subsequent to AML or non-SCC tumors, and the endpoint non-SCC Death includes deaths in AML cases who were not transplanted.

In each cohort, we estimated the cumulative incidence of each event in the presence of the competing risks using the nonparametric maximum likelihood estimator [23]. We obtained flexible and smooth estimates of the absolute cause-specific hazard functions using spline functions [24]. In SLH, we also estimated absolute non-SCC Death rates for the periods 0 – 6 months, 7 – 12 months, and > 12 months since transplant using tabulations of non-SCC deaths and person-years, and Poisson regression [25].

Risk Factor Analysis

In SLH, we examined eight treatment factors, four host factors and three donor factors for association with the outcomes non-SCC Death and development of SCC. These hypotheses were declared in our study protocol prior to analyzing the data; we describe the specific factors in Results. For each outcome, we considered these sets of risk factors as three separate families of hypotheses. We adjusted for multiple comparisons *within* each family to control the False Discovery Rate (FDR)[26]. The FDR is the expected proportion of falsely rejected hypotheses among the set of rejected hypotheses. We report both raw and FDR adjusted p-values[27]. We conducted two

complementary analyses of each hypothesis. The first analysis[28], called Rate Ratio analysis, contrasted the ratio of event rates observed in two groups using the confidence limits and p-value obtained from an exact binomial test. A potential drawback of this method is that it does not control for time, i.e., it compares two aggregate rates. The second analysis estimated the hazard ratio in two groups using the proportional hazards model, which does account for time. We report the exact (Rate Ratio) method in Results, except for the analysis of host factors and SCC, in which we used proportional hazards, because we found clear evidence of confounding by time.

Comparative Analysis of Age-Specific Event Rates

Finally, we made a comparative (cross-sectional) analysis of age-specific SCC and non-SCC Death rates in transplanted versus untransplanted FA. In this analysis, we compared rates in NAS subjects prior to BMT to corresponding rates in SLH patients who survived the high-risk peri-transplant period of 0 to 6 months and attained the same age. We excluded the peri-transplant period because it has no comparable counterpart in the non-transplanted natural history of FA. Thus, this analysis included the subset of SLH patients who survived past six months; they entered follow-up at the age at transplant plus 0.5 years, and exited follow-up at the age last known alive. From these data, we obtained smoothed estimates of age-specific hazard rates of SCC and non-SCC Death in each cohort using spline functions. We then computed the cumulative incidence of SCC by age, according to the actuarial definition; in SLH, this calculation accounted for late entry [29]. These calculations give hypothetical probabilities of development of SCC, *if the competing risk of non-SCC death could be removed*, and the risk of SCC remained unchanged.

RESULTS

In NAS, 145 untransplanted FA patients contributed 1,983 person-years, 21 non-SCC Deaths, and 7 SCC. In SLH, 117 transplanted FA patients contributed 508 person-years, 48 non-SCC Deaths, and 11 SCC (Table 1). Patients tended to be older at transplant in SLH than NAS (medians 10.8 and 8.9, P = 0.048), and they tended to have earlier birth years (medians 1981 and 1985, P = 0.003). Patients developed SCC at significantly younger ages in SLH than NAS (medians 19 and 33 years, P = 0.004).

Table 1. Characteristics of FA patients in the NAS and SLH cohorts.

	NAS	SLH	P-Value*
No. of cases	145	117	
Person-Years of follow-up	1983	508	
Male to female ratio	76:69	56:61	P = 0.54
Year of Birth, (inter-quartile range¤)	1985 (1980 – 1990)	1981 (1974 – 1987)	P = 0.003
Median age, years (inter-quartile range) at stem cell transplant	8.9 (5.7 – 12.7) [§]	10.8 (7.4 – 13.5)	P = 0.048
Total deaths prior to squamous cell cancer (SCC)	21	48	
Total SCC[#]	7	11	
Ages at onset of SCC, years	23, 27, 28, 33, 33, 44, 44	10, 16, 17, 17, 18, 19, 22, 23, 24, 28, 33	P = 0.004
Time from transplant to onset of SCC, years	N.A. [⊥]	5.5, 5.7, 7.5, 8.4, 7.0, 12.4, 9.5, 9.4, 13.8, 21.8, 14.4	

¤ The inter-quartile range includes the middle 50% of the data.
* P-value from Fisher exact test (male to female ratio) or Wilcoxon test (other contrasts).
[§] Fifty subjects in NAS had a stem cell transplant (BMT); these subjects were not followed beyond BMT in this analysis.
[#] These tumors (all head and neck cancers) arose pre-BMT in NAS and post-BMT in SLH.
[⊥] N.A. not applicable.

SCC was an adverse risk factor for death, in both the transplanted and the untransplanted cohorts. In untransplanted FA (NAS), the risk of death was increased 30-fold (95% CI = 2.9 – 317, P = 0.002) subsequent to SCC; in transplanted FA (SLH), it was increased 66-fold (95% CI = 12.3 – 352, P = $4.2*10^{-7}$). Survival after SCC was not significantly different between the two cohorts (P = 0.135); the median survival after SCC in the 18 SCC cases was 13 months.

Competing Risks Analysis in NAS and SLH

In NAS, the cause-specific hazard of development of SCC increased at a greater than linear rate (Figure 1A), approaching 4.4% per year (%/y) by age 40 (95% CI = 1.9 – 7.7%/y). The cause-specific hazard of non-SCC Death (from complications of bone marrow failure and non-SCC solid tumors) leveled off at 1.5%/y by age 13 years (95% CI = 0.8 – 2.3%/y). The cause-specific hazard of bone marrow transplant (BMT) increased to a peak of around 4.4%/y at age 7 years (95% CI = 3.2 – 5.7%/y). Using the competing risks definition for the three endpoints, the cumulative incidence by age 45 years was 19% (95% CI = 7 – 31%) for development of SCC, 22% (95% CI = 13 – 30%) for non-SCC Death, and 52% (95% CI = 39 – 64%) for BMT (Figure 1B).

In SLH, the cause-specific hazard of development of SCC also increased at a greater than linear rate, rising to 4.7%/y (95% CI = 2.1 – 8.3%/y) by 10 years after transplant and 10.1%/y (95% CI = 4.2 – 20.5%/y) by 15 years after transplant (Figure 1C). The cause-specific hazard of non-SCC Death was biphasic. The mortality rate was extremely high during the first six months after transplant (Figure 1C). Using the competing risks definition, by 10 years after transplant, the cumulative incidence was

12% for development of SCC and 44% for non-SCC Death (Figure 1D). By 15 years after transplant, the cumulative incidence of SCC rose to 24% (95% CI = 10 – 38%), and the cumulative incidence of non-SCC Death rose to 55% (95% CI = 39 – 71%).

Risk Factor Analysis in SLH

Non-SCC Death

A number of factors were significantly associated with the hazard of non-SCC Death, after controlling for multiple comparisons (Table 2). The significant treatment factors include exposure to anti-thymocyte globulin, graft versus host disease prophylaxis more intensive than cyclosporin A alone, total lymphoid irradiation, and busulphan. Interpretation of these effects is difficult because treatment factors are confounded with the underlying difficulty of the transplant. In addition, the host factors of severe acute graft versus host disease (grades III+IV – severe AGVHD – versus none through grade II) and older age at transplant were significant (adjusted p-values of $1.6*10^{-16}$ and 0.001, respectively). One donor factor, HLA alternative donors versus matched sibling donors, was significant (adjusted p-value of $1.0*10^{-8}$).

Rates of non-SCC Death during the periods 0 – 6 months, 7 – 12 months, and > 12 months since transplant are shown in Figure 2, panels B-D respectively, for the high and low risk subgroups identified in Table 2. Figure 2A shows the number of patients in each subgroup. During the period 0 – 6 months, the overall non-SCC mortality rate was 7.1% per month (%/m) (95% CI = 5.2 – 9.9%/m), ranging from 2.4 – 20.6%/m across the subgroups. Rates were uniformly lower during the period 7 – 12 months (Figure 2C), during which the overall rate was 1.1%/m (95% CI = 0.4 – 2.8%/m). The hazard was

significantly lower thereafter (P = 0.004). During this period > 12 months post BMT (Figure 2D), the overall non-SCC Death rate was 0.13%/m (95% CI = 0.064 – 0.283%/m). On an annualized basis, this rate equals 1.6%/y (95% CI = 0.8 – 3.4%/y). During the period 0 – 6 months, the lowest monthly non-SCC mortality rate, 2.4%/m, was observed in patients whose donor was an HLA-matched sibling versus all types of alternative donors (Figure 2B). After this period, the non-SCC mortality rate in this group was not lower than the overall mortality rate (Figure 2C-D). In each time period, the highest non-SCC mortality rate was observed in the 27 patients who developed severe AGVHD (Figure 2B-D). During the period > 12 months, the monthly non-SCC mortality rate in this high-risk group was 1.19%/m, significantly higher than the corresponding overall rate.

In the low-risk subgroup with an HLA-matched sibling donor, 60% were alive and free of SCC for a decade or longer after BMT, compared to 19% in the high-risk subgroup with alternative donors. In the very high-risk subgroup that developed severe AGVHD, only 4% were alive and free of SCC at six years after SCT.

Table 2. Significance analysis and relative hazards (RHs) for factors affecting the risks of non-SCC Death and development of SCC in SLH.

Factors[a]	non-SCC Death (48 events)		SCC (11 events)	
Treatment Factors	RH (95% CI)[b]	Raw P-Value[c] (Adjusted P-Value)[d]	RH (95% CI)[b]	Raw P-Value (Adjusted P-Value)
Anti-thymocyte globulin	**4.1 (2.2 – 7.3)**	**8.3×10^{-6} (6.7×10^{-5})**	0[f]	0.44
Other GVHD prophylaxis	**2.9 (1.6 – 5.1)**	**2.6×10^{-4} (0.001)**	1.8	0.51
Total lymphoid irradiation at 5Gy	**0.4 (0.2 – 0.8)**	**0.01 (0.03)**	1.9	0.90
CR with busulphan	**5.0 (1.3 – 13.6)**	**0.02 (0.04)**	0	1.00
Total body irradiation at 5Gy	2.0	0.11	0	0.69
CD34+ cell selection	1.9	0.18	0	0.87
CR with fludarabine	1.6	0.71	0	1.00
CR with cyclophosphamide	N.D.[e]	1.00	N.D.[e]	1.00
Host Factors				
Acute GVHD: grade III+IV vs. 0-II	**13.8 (7.8 – 22.2)**	**4.0×10^{-17} (1.6×10^{-16})**	**32.8 (2.7 – 392)**	**0.006 (0.026)**
Age at transplant: ≥10.8 y vs. <10.8 y	**2.7 (1.5 – 4.9)**	**5.2×10^{-4} (0.001)**	2.2	0.22
Chronic GVHD: extensive vs. none+limited; f/u beyond 6 months	2.3	0.29	**∞[f]**	**0.02 (0.03)**
Sex of patient: male vs. female	1.0	1.00	2.9	0.14
Donor Factors				
HLA: alternative donors vs. matched sibs	**5.4 (3.0 – 9.7)**	**3.3×10^{-9} (1.0×10^{-8})**	0.8	1.00
Sex of donor: male vs. female	1.8 (1.0 – 3.3)	0.052 (0.08)	1.5	0.70
Year of transplant: ≥1993 vs. <1993	1.7	0.09	0	0.11

[a] Treatment, host, and donor factors are analyzed as separate families of hypotheses, as described in methods. CR is conditioning regimen; GVHD is graft versus host disease. The median age at transplant was 10.8 y, and the median year of transplant was 1993. Other GVHD prophylaxis contrasts cyclosporin A (CSA) alone versus any of the following "other" regimens: CSA+monoclonal antibody, steroids, CSA+steroids, methotrexate, CSA+methotrexate, CSA+anti-thymocyte globulin+steroids. Total lymphoid irradiation (TLI) and total body irradiation (TBI) contrast TLI and TBI versus no TLI and no TBI, respectively.

[b] Relative hazard (RH) and exact 95% Confidence Interval (CI) and p-value estimated by Rate Ratio analysis, except for analyses of host factors and SCC. In those analyses, RHs were obtained using the proportional hazards models, because time was associated with the risk factors acute and chronic GVHD and age at transplant, and with the outcome SCC.

[c] P-values unadjusted for multiple comparisons.

[d] P-values adjusted for multiple comparisons using the Benjamini-Hochberg FDR procedure are shown whenever the raw p-values are less than or nearly equal to 0.05. The estimates shown in bold are statistically significant using the FDR criterion.

[e] N.D. not determined; in 117 patients, 113 received cyclophosphamide and 4 did not. Rate Ratio analysis was not informative.

[f] An RH of ∞ is obtained if all 11 SCC events occur in a subgroup of interest. An RH of 0 is obtained if none of the 11 SCC events occur in a subgroup.

Development of SCC

Two factors were significantly associated with the hazard of development of SCC, after controlling for multiple comparisons (Table 2). Severe AGVHD was associated with a 33-fold increase in the hazard of SCC (95% CI = 2.7 – 392, adjusted P = 0.026). Chronic graft versus host disease, extensive versus limited or none (CGVHD), was also significantly associated with SCC (adjusted P = 0.03); all 11 patients with SCC had previously developed CGVHD. Severe AGVHD remained as a significant risk factor for SCC in a proportional hazards model restricted to the 41 patients with CGVHD (P = 0.002). Among 41 patients with CGVHD, 8 patients had severe AGVHD, 2 of whom developed SCC. These two tumors had the shortest observed latency periods (5.5 and 5.7 years) and the youngest ages at onset (10 and 16 years) (Table 1).

Comparative Analysis of Age-Specific Event Rates

We contrasted age-specific event rates in NAS (Table 1) with the corresponding rates in SLH patients who survived the peri-transplant period (defined as 0 to 6 months) and attained the same age. Sixty-five SLH patients followed beyond the peri-transplant period contributed 464 person-years, 11 non-SCC Deaths, and 11 SCC to this comparative analysis. In SLH, the age-specific hazard of development of SCC increased at a greater than linear rate (Figure 3A). The shape of the hazard function in SLH was similar to that observed in NAS. By age 25 years, the annual hazard for SCC in SLH was 6.2%/y (95% CI = 2.9 – 13.0%/y). The annual hazard for SCC within the NAS reached similar levels much later in life, i.e., 6.3%/y (95% CI = 2.3 – 11.2%/y) by age 45 years, indicating that SLH patients attained high hazard rates at considerably younger ages than

NAS patients. In a proportional hazards model, the age-specific hazard of SCC was 4.4-fold higher in SLH compared to NAS (95% CI = 1.7 – 11.9, P = 0.003).

Rates of non-SCC Death were slightly higher in younger SLH patients compared to NAS subjects of the same age (Figure 3B). The death rates appeared progressively higher in older SLH patients, however, the estimated hazard rates are uncertain due to the small sample size. In a proportional hazards model, the overall hazard ratio (HR) was not significantly elevated in SLH patients (HR = 1.5, 95% CI = 0.7 – 3.3, P = 0.30). A test of cohort-by-time interaction was marginally significant (P = 0.053).

In each cohort, we computed the cumulative incidence of SCC by age according to the actuarial definition that "removes" the competing risk of non-SCC Death (Figure 4). In this scenario, 50% of transplanted patients are projected to develop SCC by age 29 years, whereas 50% of untransplanted patients are expected to develop SCC by age 45 years. The projected 16-year shift in the age-at-onset distribution of SCC is consistent with the younger ages-at-onset of SCC observed in SLH versus NAS in the presence of competing mortality (Table 1).

DISCUSSION

Stem Cell Transplantation for Fanconi Anemia presents a challenging therapeutic balancing act [8]. The conditioning regimen must be strong enough to enable engraftment, but not so strong that it kills the host. Standard regimens using high doses of irradiation and cyclophosphamide are too toxic for FA patients, who are hypersensitive because of their underlying DNA repair defect. SCT regimens that are modified for FA patients have met with some success. Overall, 44% of SLH patients were alive and free of SCC for a decade or longer after SCT. This probability was increased to 60% among the subset of patients with an HLA-identical sibling donor. In contrast, 96% of patients who developed severe AGVHD had died or developed an SCC by six years after SCT. Overall, the rate of non-SCC Death was similar in transplanted patients who survived to the six-month landmark compared to untransplanted patients of the same age, with a non-significant trend towards higher mortality rates in long-term transplant survivors.

The non-SCC Death rates observed here for the entire SLH cohort, and for high and low risk subgroups, might serve as historical control rates in power and sample size calculations for new transplant protocols, and to assess the evolving experience with newer transplant regimens in use today. It must be emphasized that technologies in use now may not result in such a high incidence of SCC. Furthermore, as previously recognized [30], many of the risk factors are inter-dependent, and we did not attempt to separate the effects. This makes it difficult to rigorously compare an event rate observed in a heterogeneous cohort of patients to a single historical control rate. Nonetheless, rough comparisons might be useful when it is not feasible to conduct randomized clinical trials.

Hopefully, new conditioning regimens will improve the outcomes by reducing the trio of hazards described in our analyses: early deaths, late deaths, and SCC arising years after SCT. In both the transplanted and untransplanted cohorts, the prognosis was similarly poor after SCC; it can be difficult to treat these tumors using standard radiation and chemotherapy regimens because FA patients are particularly sensitive to their cytotoxic effects. [31].

In the SLH cohort, severe AGVHD (grades III+IV versus grades none through grade II) was a strong risk factor, both for non-SCC mortality and for the development of SCC. CGVHD (extensive versus limited plus none) was also a strong risk factor for SCC. Indeed, all SLH patients who developed SCC had CGVHD. Severe AGVHD and CGVHD may have independent effects on SCC. The current analysis provides additional insight into the previously reported association between AGVHD and the risk of adverse outcomes in a subset of SLH patients who received transplants from matched sibling donors [20].

A minority of patients, 18 percent, was transplanted using T-cell depletion of the stem cell source via CD34+ stem cell selection. A larger minority of patients, 35 percent, was conditioned using ATG. As a group, SLH patients might have had a comparatively high risk of AGVHD and CGVHD compared to cohorts of patients whose conditioning regimens included both of these modalities [32-34]. However, T-cell depletion and/or ATG may also increase the risk of graft failure [34,35]. In our view, the optimal protocol remains unclear. The SLH group does not use ATG with matched sibling donors but does use ATG with unrelated donors. By this point in time, several other transplant centers may

have long-term follow-up of substantial numbers of patients treated with T-cell depletion and/or ATG, and a similar study of the long-term outcomes would be of interest.

We compared the rate of development of SCC in transplanted FA patients to the rate in untransplanted FA patients of the same age. We found that the rate was 4.4-fold higher after SCT, a significant elevation. This relative risk is of the same order as that observed in heterogeneous cohorts of non-FA patients transplanted for a number of indications [9-14]. Cyclophosphamide and irradiation are thought to be independent transplant-related risk factors for SCC [30]. FA patients are hypersensitive to each of these exposures, and for this reason, they receive comparatively low doses to compensate [36]. On balance, the *relative* risks observed in transplanted FA patients compared to untransplanted FA patients are similar to those in transplanted non-FA patients compared to the general population. However, this elevated relative risk is acting on the high Fanconi baseline. The absolute cause-specific hazard rate of SCC increased over time, to remarkably high values: 4.7%/y by 10 years after transplant, and 10.1%/y by 15 years after transplant. In the presence of competing mortality, the cumulative incidence of SCC was 24% by 15 years after transplant. As shown in our comparative analysis, SCC manifests at considerably younger ages in transplanted than in untransplanted FA patients.

Strengths of our study include the representativeness of each cohort, the duration of follow-up, and our analytical methods. These allowed us to develop competing risks models for each cohort, identify significant risk factors despite limited numbers of events, and contrast the experience of each cohort while accounting for the later ascertainment of patients in SLH beginning at the age at transplant.

The major limitation of our study is that we cannot prove that subjects in the NAS and the SLH belonged to cohorts that were born with the same intrinsic susceptibility to SCC. The NAS cohort consists of 145 respondents out of 318 subjects who belonged to the United States and Canadian FA family support groups. Therefore, the cohort is subject to the bias of volunteerism, and may under-estimate cancer incidence. However, since it was known to be a cancer study, it could over-estimate cancer incidence. It is reassuring that the cancer risks pre-transplant in the NAS, in the literature, and in the IFAR are all similar [37]. In addition, most of the North American patients are of European origin, and thus somewhat comparable with respect to ethnic background to the patients transplanted in Paris. In the absence of a comprehensive national registry or cohort of FA patients enrolled at diagnosis, these observations support our use of the NAS cohort as a "control" group for the SLH transplant cohort.

Furthermore, although we can't be sure, we do not think the NAS cohort represents a group of FA patients at substantially *lower* risk of myelodysplastic syndrome, AML, or BMT than the population treated by SLH. Indeed, as we showed previously in the NAS [4], the cumulative incidence by age 48 of BMT, AML, or non-cancer death was 64%. Thus, about two-thirds of the NAS cohort either received a BMT or developed a positive indication for one.

Since complementation group assignment and mutation testing are incomplete in both cohorts, we did not attempt to compare the genetic distributions or adjust for genetic factors. We anticipate that future studies will assemble larger FA cohorts with additional data; our analytical approaches will remain entirely applicable.

A key etiological question is whether the tumors were caused by the underlying condition of FA, by transplant-related factors, or by an interaction [30,38]. The hazard of SCC appears to increase at a greater than linear rate with the time-since-transplant, similar to the pattern seen for SCC in non-FA transplant populations treated with similar conditioning regimens [12]. Considering the young ages at onset, it seems plausible (but cannot be proven from these data) that transplant-related factors initiated or accelerated SCC in some cases.

As previously noted, both acute and chronic graft versus host diseases were strong risk factors for SCC. Two previous studies [35,39] suggested that the presence of urogenital and/or renal malformations were associated with an increased risk of acute graft versus host disease, which in turn predisposes to SCC. The biological basis for this association is unclear, and we did not examine this finding in our cohort.

Novel "milder" SCT conditioning regimens for FA patients have recently been designed, which employ less or no cyclophosphamide, less or no irradiation, T-cell depletion, and alternative agents such as fludarabine and anti-thymocyte globulin [32,33,40-42]. One goal of these protocols is to reduce both acute and chronic graft versus host diseases. Hopefully, these gentler regimens will also result in lower rates of SCC and non-SCC Death, but patients treated with these new modalities must be carefully monitored (long-term) for unexpected adverse events [43,44].

Despite past progress and the potential of new SCT protocols to improve the outcomes, our comparative analysis highlights an important and discouraging fact. Even if an optimal SCT protocol could be developed that eliminated non-SCC deaths and reduced SCC incidence to baseline, one would still expect to see half or more of FA

patients develop SCC or another solid tumor by their mid-forties. Clearly, new interventions are needed to reduce SCC incidence in *both* transplanted *and* untransplanted patients with FA. For example, FA patients may be particularly susceptible to human papillomavirus (HPV)-induced carcinogenesis [45]. HPV vaccines, which are currently under development, might help to prevent HPV infection in both the cervix and the oropharynx [46]. Until such time, it is clear that patients with FA require meticulous surveillance for head and neck cancer, beginning at very young ages.

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FIGURE LEGENDS

Figure 1. Competing risks analysis in NAS and SLH. (A) Annual hazard rates (incidence rate per year among subjects who are still susceptible) of bone marrow failure leading to bone marrow transplant (BMT), non-SCC Death, and development of SCC, by age, in NAS, and 95% point-wise confidence envelopes (shaded regions). (B) Cumulative incidence (cumulative percent experiencing each event as initial cause of failure, in subjects at risk of each adverse event) by age, in NAS, and 95% CIs at selected years (error bars). (C) Annual hazard rates of non-SCC Death and development of SCC, in SLH, by years since transplant, and 95% point-wise confidence envelopes (shaded regions). (D) Cumulative incidence, by years since transplant, in SLH, and 95% CIs at selected years. Hazard rates shown in (A) and (C) are plotted using different y-axis scales. In (C), corresponding *crude* monthly non-SCC death rates for months 1 to 6 were: 7.1, 10.7, 9.1, 7.6, 5.7, and 0%/m, respectively.

Figure 2. Monthly non-SCC death rates in subgroups of SLH patients. Estimates are shown for factors found to be significant in Table 2. ATG is anti-thymocyte globulin, TLI is total lymphoid irradiation, and BU is busulphan. (A) Number of patients in each subgroup. (B) Incidence rate per month of non-SCC Death during the period 0 – 6 months since transplant, by subgroup, and 95% confidence intervals (error bars) obtained using Poisson regression. Reference line and interval (shaded) shows the monthly death rate and its 95% confidence interval for the entire SLH cohort. (C) Incidence rates per month of non-SCC Death during the period 7 – 12 months since transplant, overall and by subgroup. (D) Incidence rates per month of non-SCC Death during the period > 12

months since transplant, overall and by subgroup. No TLI- or BU+ subjects were followed beyond 6 months, so the corresponding rates in C and D cannot be determined.

Figure 3. Comparative annual hazard rates by age in NAS and SLH. (A) Annual hazard rates of SCC in NAS and SLH, by age, and 95% confidence envelopes (shaded regions). (B) Annual hazard rates of non-SCC Death in NAS and SLH, by age, and 95% confidence envelopes. Comparative SLH hazard rates in (A) and (B) were derived using data from patients who survived beyond the six month landmark.

Figure 4. Hypothetical cumulative incidence curves for SCC in NAS and SLH. Observed actuarial cumulative incidence curves for SCC (step functions), and spline-smoothed estimates (smooth curves); shaded regions show 95% point-wise confidence intervals. Curves in NAS and SLH indicate the cumulative incidence of SCC expected if the competing risks of non-SCC Death could be removed and the hazard of development of SCC remained unchanged.

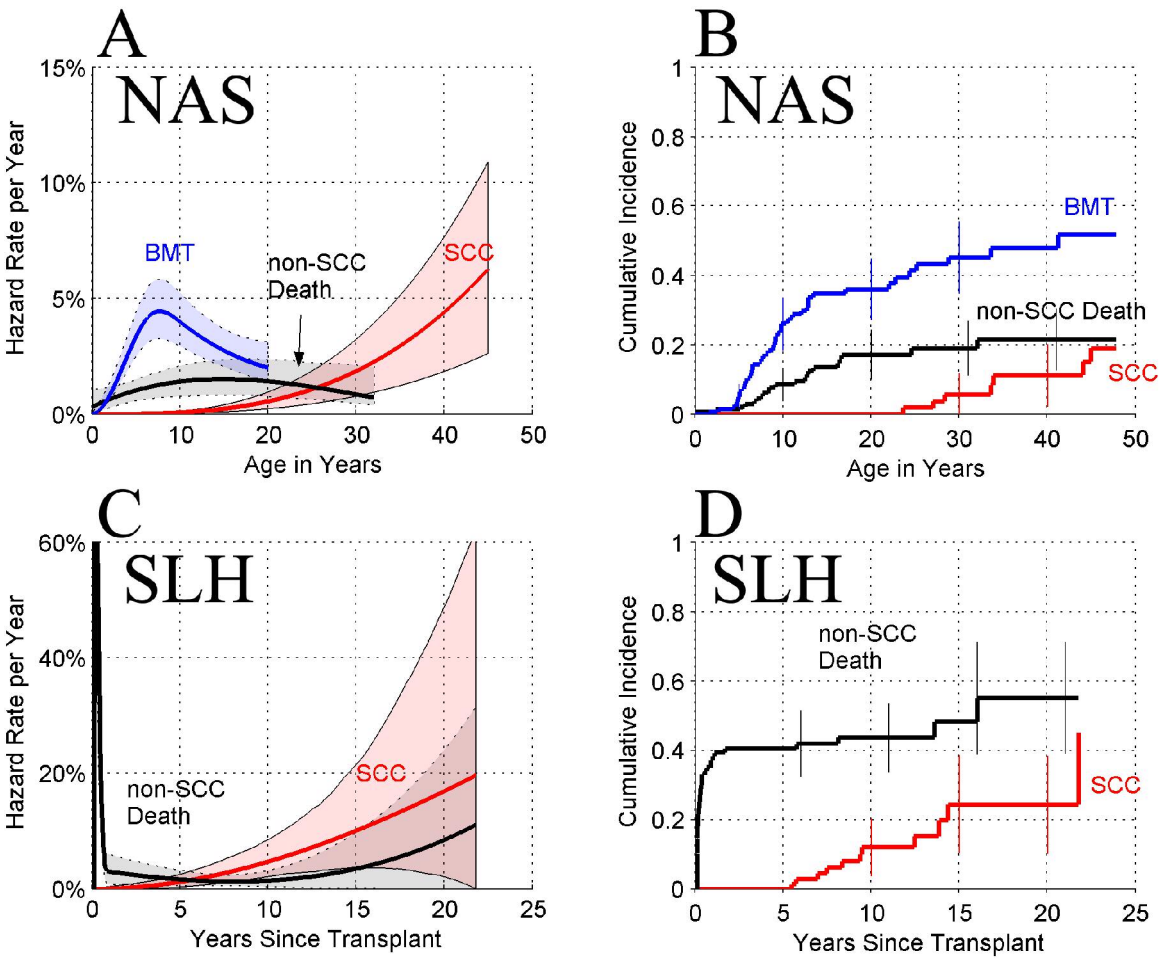
A
NAS
Hazard Rate per Year
15%
10%
5%
0%
BMT
non-SCC
Death
SCC
0
10
20
30
40
50
Age in Years
B
NAS
Cumulative Incidence
1
0.8
0.6
0.4
0.2
0
BMT
non-SCC Death
SCC
0
10
20
30
40
50
Age in Years
C
SLH
Hazard Rate per Year
60%
40%
20%
0%
non-SCC
Death
SCC
0
5
10
15
20
25
Years Since Transplant
D
SLH
Cumulative Incidence
1
0.8
0.6
0.4
0.2
0
non-SCC
Death
SCC
0
5
10
15
20
25
Years Since Transplant

Fig. 1.

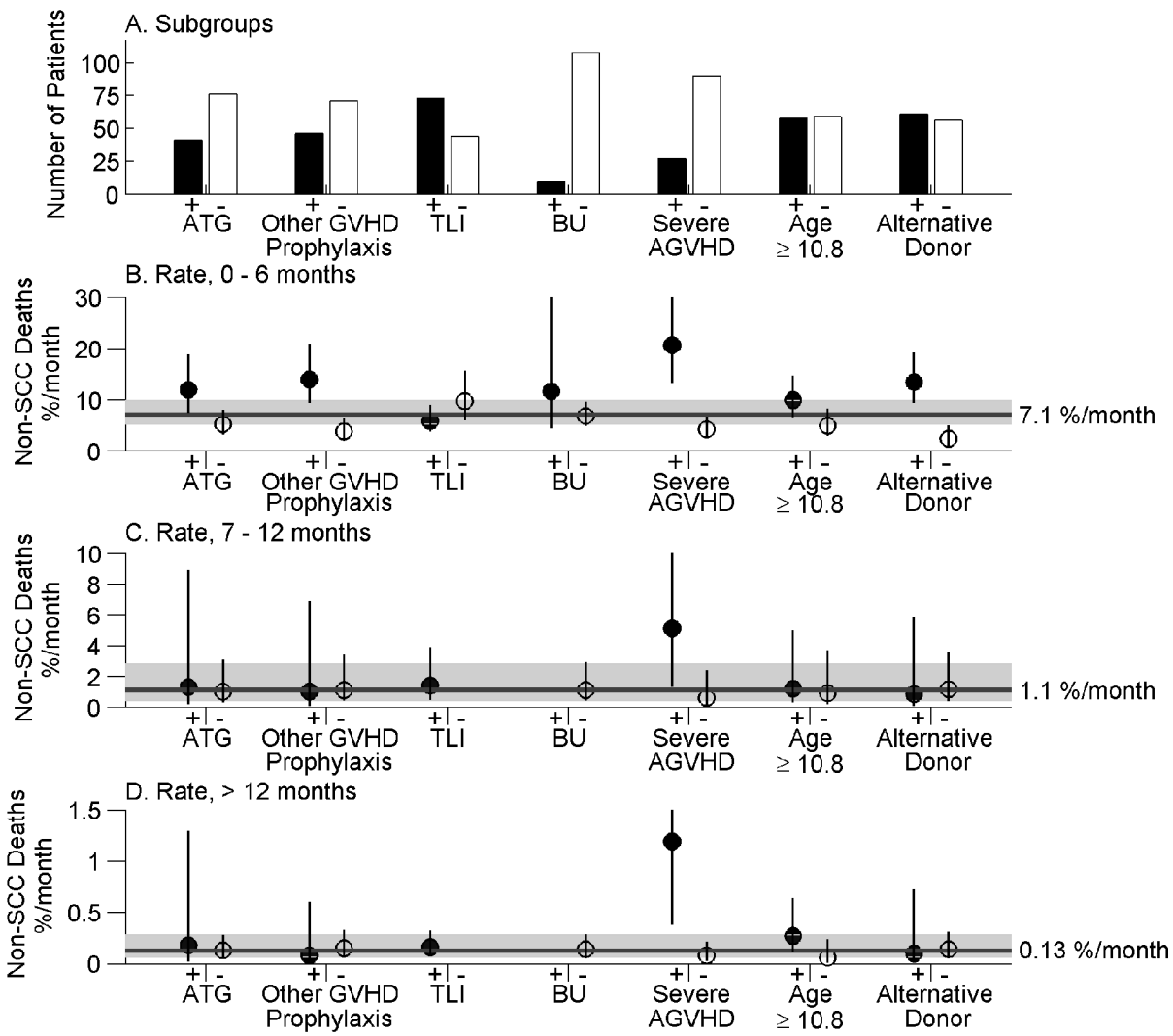
A. Subgroups
Number of Patients
100
75
50
25
0
+ -
ATG
Other GVHD Prophylaxis
TLI
BU
Severe AGVHD
Age ≥ 10.8
Alternative Donor
B. Rate, 0 - 6 months
Non-SCC Deaths %/month
30
20
10
0
7.1 %/month
C. Rate, 7 - 12 months
10
8
6
4
2
0
1.1 %/month
D. Rate, > 12 months
1.5
1
0.5
0
0.13 %/month

Fig. 2.

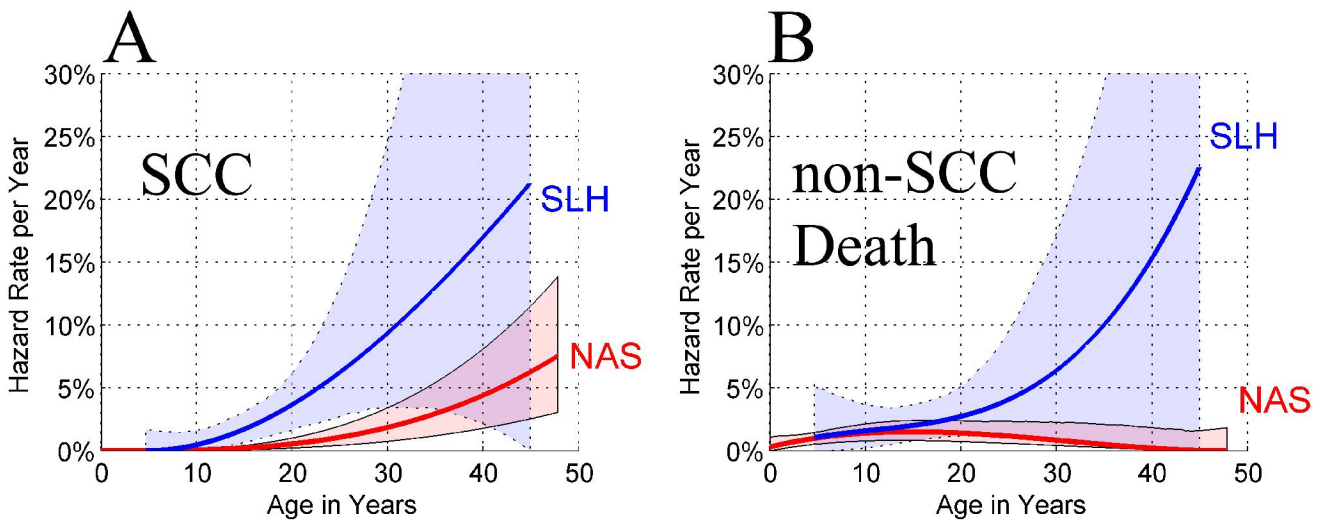
A
SCC
SLH
NAS
Hazard Rate per Year
30%
25%
20%
15%
10%
5%
0%
0
10
20
30
40
50
Age in Years
B
non-SCC
Death
SLH
NAS
Hazard Rate per Year
30%
25%
20%
15%
10%
5%
0%
0
10
20
30
40
50
Age in Years

Fig. 3.

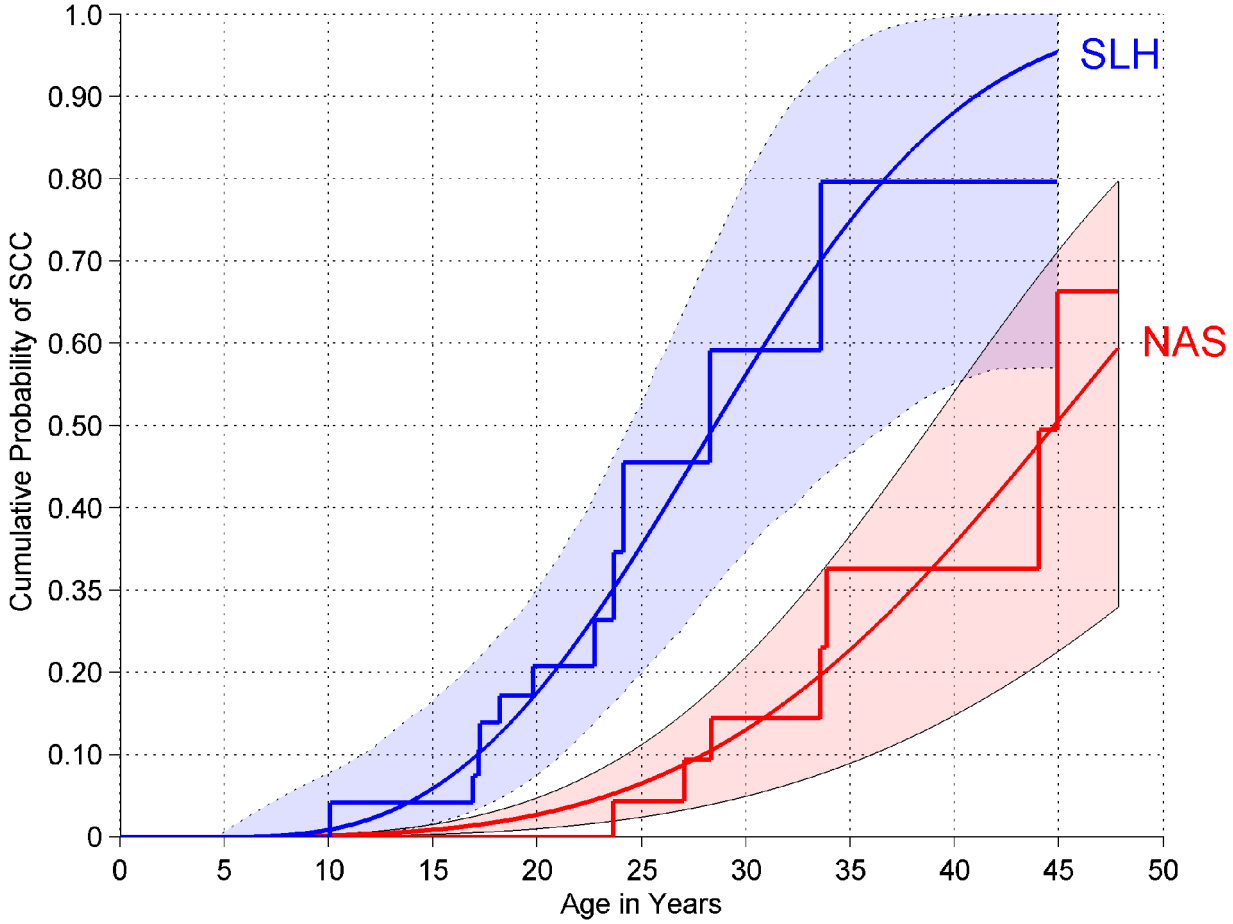
1.0
0.90
0.80
0.70
0.60
0.50
0.40
0.35
0.20
0.10
0
Cumulative Probability of SCC
0
5
10
15
20
25
30
35
40
45
50
Age in Years
SLH
NAS

Fig. 4.

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